SEQUENCE LISTING

<110> Xia, Zhi-Qiang
 Costa, Michael A
 Davin, Laurence B
 Lewis, Norman G

<120> Recombinant Secoisolariciresinol Dehydrogenase, and Methods of Use

<130> WSUR116430

<140> 09/673,918

<141> 2000-10-23

<150> PCT/US99/08975

<151> 1999-04-23

<150> 60/082,977

<151> 1998-04-24

<160> 25

<170> PatentIn Ver. 2.0

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<211> 819

<212 > DNA

<213> Forsythia x intermedia

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ctt ata aca gga gga gcc agt gga att gga gaa acc aca gca aaa ctc 96 Leu Ile Thr Gly Gly Ala Ser Gly Ile Gly Glu Thr Thr Ala Lys Leu 20 25 30

ttc tcc caa cat gga gcc áaa gtt gcc att gct gat gtc caa gat gaa 144 Phe Ser Gln His Gly Ala Lys Val Ala Ile Ala Asp Val Gln Asp Glu 35 40 45

tta ggt cac tca gtt gtc gag gcc att ggc act tcc aat tcc acc tac 192 Leu Gly His Ser Val Val Glu Ala Ile Gly Thr Ser Asn Ser Thr Tyr 50 55 60

atc cac tgt gat gtt act aat gaa gac ggt gtt aaa aat gcc gtg gac 240 Ile His Cys Asp Val Thr Asn Glu Asp Gly Val Lys Asn Ala Val Asp

70 75 . aac aca gtt tca acc tat gga aaa ctg gac att atg ttc agc aat qca Asn Thr Val Ser Thr Tyr Gly Lys Leu Asp Ile Met Phe Ser Asn Ala gga att tet gat eec aac agg eec ege ate ata gae aac gaa aaa gea Gly Ile Ser Asp Pro Asn Arg Pro Arg Ile Ile Asp Asn Glu Lys Ala 100 105 gac ttt gaa ege gtt ete agt gta aat gta ace gga gtt tte eta tge 384 Asp Phe Glu Arg Val Leu Ser Val Asn Val Thr Gly Val Phe Leu Cys 115 atg aag cac gca gca cgt gtt atg att cca gca cgc agt ggc aac ata 432 Met Lys His Ala Ala Arg Val Met Ile Pro Ala Arg Ser Gly Asn Ile 135 130 att tee act get agt tta age tea act atg ggt ggt tet tea cat 480 Ile Ser Thr Ala Ser Leu Ser Ser Thr Met Gly Gly Ser Ser His 150 155 gcc tat tgt ggt tca aag cat gct gtg tta gcc ctt act agg aat ctg 528 Ala Tyr Cys Gly Ser Lys His Ala Val Leu Ala Leu Thr Arg Asn Leu 165 gca gtc gag ctc gga caa ttt ggc att agg gtt aat tgt ttg tct cct 576 Ala Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn Cys Leu Ser Pro 180 185 ttc ggg ctt cct acg gct tta ggc aag aaa ttt tca ggg att aaa aat 624 Phe Gly Leu Pro Thr Ala Leu Gly Lys Phe Ser Gly Ile Lys Asn 195 200 gaa gaa gaa ttt gag aat gta ata aac ttt gcg gga aat ttg aaa ggt Glu Glu Glu Phe Glu Asn Val Ile Asn Phe Ala Gly Asn Leu Lys Gly 210 215 cca aaa ttt aat gtt gag gat gtt gcc aat gca gct ctt tat ctg gct Pro Lys Phe Asn Val Glu Asp Val Ala Asn Ala Ala Leu Tyr Leu Ala 225 230 agt gat gag gea aaa tac gtg agt gga cac aat ctg ttc att gat gga 768 Ser Asp Glu Ala Lys Tyr Val Ser Gly His Asn Leu Phe Ile Asp Gly 245 250 ggg ttc agc gtc tgc aat tct gta atc aaa gtg ttc caa tat cca gat Gly Phe Ser Val Cys Asn Ser Val Ile Lys Val Phe Gln Tyr Pro Asp 260 265 tct 819 Ser

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Leu Gly His Ser Val Val Glu Ala Ile Gly Thr Ser Asn Ser Thr Tyr 50 55 60

Ile His Cys Asp Val Thr Asn Glu Asp Gly Val Lys Asn Ala Val Asp 65 70 75 80

Asn Thr Val Ser Thr Tyr Gly Lys Leu Asp Ile Met Phe Ser Asn Ala 85 90 95

Gly Ile Ser Asp Pro Asn Arg Pro Arg Ile Ile Asp Asn Glu Lys Ala 100 105 110

Asp Phe Glu Arg Val Leu Ser Val Asn Val Thr Gly Val Phe Leu Cys 115 120 125

Met Lys His Ala Ala Arg Val Met Ile Pro Ala Arg Ser Gly Asn Ile 130 135 140

Ile Ser Thr Ala Ser Leu Ser Ser Thr Met Gly Gly Gly Ser Ser His 145 150 155 160

Ala Tyr Cys Gly Ser Lys His Ala Val Leu Ala Leu Thr Arg Asn Leu 165 170 175

Ala Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn Cys Leu Ser Pro 180 185 190

Phe Gly Leu Pro Thr Ala Leu Gly Lys Lys Phe Ser Gly Ile Lys Asn 195. 200 205

Glu Glu Glu Phe Glu Asn Val Ile Asn Phe Ala Gly Asn Leu Lys Gly 210 215 220

Pro Lys Phe Asn Val Glu Asp Val Ala Asn Ala Ala Leu Tyr Leu Ala 225 230 235 240

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Gly Phe Ser Val Cys Asn Ser Val Ile Lys Val Phe Gln Tyr Pro Asp 260 265 270

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•gga aaa gtt	gcc ctt at	a aca gga	gga gcc	agt gga a	itt gga ga	aa acc 96
Gly Lys Val						
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	*					
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gtc caa gat						
Val Gln Asp	Giu Leu G.	=	val val		te Giy Tr	ır Ser
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aat tcc acc	tac atc ca	c tot cat	att act	aat daa d	ec aat at	t aaa 240
Asn Ser Thr						
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Phe Ser Asn		e Ser Asp.		Arg Pro A		.e Asp
	100	-	105		110	
aac gaa aaa						
Asn Glu Lys 115	AIA ASP P	ie Glu Arg 120	•			ir GIA
. 112		120	•		.25 ,	•
gtt ttc cta	Itac ata as	מ כפר מכפ	aca cat	att ata a	tt cca.co	a cgc 432
Val Phe Leu						
.a. Inc bea	cio mos mi	· HILD ALG	Ara Ary	AGT LICE T	TC FIG MI	.u Ary

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ag	t ggc	aac	ata	att	tcc	act	gct	agt	tta	agc	tca	act	atg	ggt.	ggt	480
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GT	y Ser	ser	HIS	165	Tyr	Cys	GIY	Ser		His	Ala	Val	Leu		Leu	
				100	·	•			170					175	•	•
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	r Arg													_		
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СУ	s Leu		Pro	Pne	GTA	Leu		Thr	Ala	Leu	Gly	_	Lys	Phe	Ser	
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	u Tyr															700
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	c.att	_				_	_	_			_					816
Ph	e Ile	Asp		Gly	Phe	Ser	Val	Cys	Asn	Ser	Val	Ile	Lys	Val	Phe	
			260			•		265					270		•	
C a	a tat	CCa	ant.	+ ~ +									,		* .	831
_	n Tyr		_													03T
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	t Ala	Ala	Thr	Ser	Gln	Val	Leu	Thr	Ala	Ile	Ala	Arg	Arg	Leu	Glu	
	l			5		•	*		10				Ĭ.	15		`
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Gl	y Lys	Val	Ala	Leu	Ile	Thr	Gly	Gly	Ala	Ser	Gly	Ile	Gly	Glu	Thr	
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1111	r Ala	тÀг	ьeu	FIIE	ser	GIN	H15	GTA	нта	ьys	val	ATG	тте	ALA	Asp	,

Val Gln Asp Glu Leu Gly His Ser Val Val Glu Ala Ile Gly Thr Ser 50 55 60

Asn Ser Thr Tyr Ile His Cys Asp Val Thr Asn Glu Asp Gly Val Lys
65 70 75 80

Asn Ala Val Asp Asn Thr Val Ser Thr Tyr Gly Lys Leu Asp Ile Met 85 90 95

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115 120 125

Val Phe Leu Cys Met Lys His Ala Ala Arg Val Met Ile Pro Ala Arg 130 135 140

Ser Gly Asn Ile Ile Ser Thr Ala Ser Leu Ser Ser Thr Met Gly Gly 145 150 155 160

Gly Ser Ser His Ala Tyr Cys Gly Ser Lys His Ala Val Leu Gly Leu 165 170 175

Thr Arg Asn Leu Ala Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn 180 185 190

Cys Leu Ser Pro Phe Gly Leu Pro Thr Ala Leu Gly Lys Lys Phe Ser 195 200 205

Gly Ile Lys Asn Glu Glu Glu Phe Glu Asn Val Ile Asn Phe Ala Gly 210 215 220

Asn Leu Lys Gly Pro Lys Phe Asn Val Glu Asp Val Ala Asn Ala Ala 225 230 235 240

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	•	^	aa =	any	amı	поа	CIG			•								
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	1	GIII	ьеи	Arg	5	Ala	TTE	ALA	Arg	Arg 10	ьeu	GIU	GIA	ьys	. 15	Ala		
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	Leu	Ile	Thr		Gly	Ala	Ser	Gly		Gly	Glu	Val	Thr		Lys	Leu		
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	пеп	50	птэ	261	, vai	vai	55	AIA	116	GIY	PIO	60	ASII	SEL	1111	TAT		
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	Ų,					, ,				• •	, 5					00		
	aac								_	-		-				_	288	
	Asn	Thr	Val	Ser	Thr 85	Tyr	Gly	Lys	Leu	Asp 90	Ile	Met	Phe	Asn	Asn 95	Ala		•
					. 03	*		:										
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	Gly	Ile	Ser	_		Tyr	Lys	Pro		Val	Ile	Asp	Asn		Lys	Ala	•	
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·	gac	ttt	gaa	cgc	gtt	ctc	agt	gtn	aat	gtn	acc	gga	gtt.	ttc	cta	ttt	384	
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	atq	aaq	cac	qca	qca	cac	att	atq	att	cca	qca	cga	aat	: aac	tac	ata	432	
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		130			•		135					140						
	att	tcc	act	qct	agt	tta	agc.	tca	act	atg	gat	aat	gat	tct	tca	cat	480	Y.
										Met		-					3	
	145	,				150					155					160		•
	act.	tat	tat	aat	ac=		cat	ac+	at =	tta		c++	a.c.t	200	a	cta	528	
		-								Leu						_	. 520	
	-	•	-	•	165	-				170	4			2	175			

Ala	gtc Val															5.76
	Gly			_			-	_						_		624
	gta Val 210															672
	aaa Lys															720
	gat Asp															768
	ttc Phe	_	_	_			_			_					Ξ.	816
tct Ser																819
DCI																
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	0 > 6	73		•										·		•
<21	0 > 6 1 > 2 2 > PF		•						ē						•	
<21 <21	1> 27	RT	chia	x iı	ntern	nedia	a									
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<211 <211 <221 <221 <222 <222 <222 <400 Met	1 > 27 2 > PF 3 > Fc 0 > 1 > mi 2 > (1 3 > Sc 0 > 6 Gln	ecoi Leu	Eeatu (273) sola Arg	rici Thr 5	resi Ala	nol (dehy Ala	Arg	Arg 10	Leu	Glu	Gly	Lys	Val 15	Ala	acid
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<21: <21: <22: <22: <22: <22: <40: Met 1 Leu Phe	1 > 27 2 > PP 3 > Fo 0 >	isc_iisc_ii)ecoi Leu Thr	Eeatu (273) sola Arg Gly 20	Thr 5 Gly	resi Ala Ala Ala	nol (Ile Ser Lys	dehy Ala Gly Val 40	Arg Val 25 Ala	Arg 10 Gly Ile	Leu Glu Ala	Glu Val Asp	Gly Thr Val 45	Lys Ala 30 Gln	Val 15 Lys Asp	Ala Leu Glu	acid

Ile His Cys Asp Val Thr Asn Glu Asp Gly Val Lys Asn Ala Val Asp

65 70 80 Asn Thr Val Ser Thr Tyr Gly Lys Leu Asp Ile Met Phe Asn Asn Ala 85 Gly Ile Ser Asp Pro Tyr Lys Pro Arg Val Ile Asp Asn Glu Lys Ala 100 105 Asp Phe Glu Arg Val Leu Ser Xaa Asn Xaa Thr Gly Val Phe Leu Phe 120 Met Lys His Ala Ala Arg Ile Met Val Pro Ala Arg Asn Gly Cys Ile . 135 Ile Ser Thr Ala Ser Leu Ser Ser Thr Met Gly Gly Ser Ser His 150 Ala Tyr Cys Gly Ala Lys His Ala Val Leu Gly Leu Thr Arg Asn Leu Ala Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn Cys Leu Ser Pro 180 185 Phe Gly Leu Pro Thr Pro Leu Ala Lys Lys Phe Ser Gly Ile Glu Asn Asp Val Asp Phe Ala Asn Ala Ile Glu His Ala Gly Asn Leu Lys Gly 215 Thr Lys Leu Arg Ile Glu Asp Val Ala Asn Ala Ala Leu Phe Leu Ala 225 230 235 Ser Asp Glu Ala Gln Tyr Val Ser Gly Gln Asn Leu Phe Ile Asp Gly 250 Gly Phe Ser Val Cys Asn Ser Ala Ile Lys Met Phe Gln Tyr Pro Asp 260 265

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<213> Forsythia x intermedia

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<222> (1)..(831)

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												gga Gly					96
												gtt Val					144
												gcc Ala 60					192
																aaa Lys 80	
							_					aaa Lys	_	_		~	288
												ccc Pro					336
												gta Val					384
•									_	_		atg Met 140			_	_	432
												tca Ser					480
												gct Ala					528
												ggc Gly					576
												gcc Ala				gta Val	624
	ggg Gly	ctt Leu	gaa Glu	aat Asn	gac Asp	gaa Glu	gat Asp	ttg Leu	gag Glu	aat Asn	gca Ala	atg Met	agc Ser	ctt Leu	atg Met	gga Gly	672

210 220 aat ctg aaa ggt aca aat ttg aag gct gag gac gtc gcc aat gca qct Asn Leu Lys Gly Thr Asn Leu Lys Ala Glu Asp Val Ala Asn Ala Ala 225 235 ctt tat ctg gca agt gat gag gca aaa tat gtg agt gga cac aat ctg Leu Tyr Leu Ala Ser Asp Glu Ala Lys Tyr Val Ser Gly His Asn Leu 245 250 ttc att gat gga ggg ttc agc gtc tac aat tct gca atc aaa atg ttc Phe Ile Asp Gly Gly Phe Ser Val Tyr Asn Ser Ala Ile Lys Met Phe 260 265 caa tat cca gac act 831 Gln Tyr Pro Asp Thr 275 <210> 8 <211> 277 <212> PRT <213> Forsythia x intermedia Met Ala Ser Thr Ser Gln Val Leu Thr Ala Ile Thr Arg Arg Leu Glu 10 Gly Lys Val Ala Leu Ile Thr Gly Gly Ala Ser Gly Ile Gly Glu Phe Thr Ala Lys Leu Phe Ser Gln His Gly Ala Lys Val Ala Ile Ala Asp Val Gln Asp Glu Leu Gly His Ser Val Val Glu Ala Ile Gly Thr Ser . 55 Asn Ser Ile Tyr Ile His Cys Asp Val Thr Asn Glu Asp Asp Val Lys 70 .75 Asn Ala Val Asp Asn Thr Val Ser Thr Tyr Gly Lys Leu Asp Ile Met 85 90 Phe Asn Asn Ala Gly Ile Ala Asp Pro Asn Lys Pro Arg Ile Val Asp 105 Asn Glu Lys Ala Asp Phe Glu Arg Val Leu Ser Val Asn Val Thr Gly 115 120 Val Phe Leu Cys Met Lys His Ala Ala Arg Val Met Val Pro Ala Arg 135

Ser Gly Ser Ile Ile Ser Thr Ala Ser Val Ser Ser Thr Ile Gly Gly

150

155

160

145

Ala Ala Ser His Ala Tyr Cys Cys Ser Lys His Ala Val Leu Gly Leu 170 Thr Arg Asn Leu Ala Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn 180 185 Cys Leu Ala Pro Tyr Ala Leu Ala Thr Pro Leu Ala Lys Lys Phe Val Gly Leu Glu Asn Asp Glu Asp Leu Glu Asn Ala Met Ser Leu Met Gly 215 220 Asn Leu Lys Gly Thr Asn Leu Lys Ala Glu Asp Val Ala Asn Ala Ala Leu Tyr Leu Ala Ser Asp Glu Ala Lys Tyr Val Ser Gly His Asn Leu Phe Ile Asp Gly Gly Phe Ser Val Tyr Asn Ser Ala Ile Lys Met Phe 265 Gln Tyr Pro Asp Thr 275 <210> 9 <211> 828 <212> DNA <213> Forsythia x intermedia <220> <221> CDS -<222> (1)..(828) <400> 9 atg gcc act tca cag ctt cga act gca ttc gca aga agg cta gaa gga Met Ala Thr Ser Gln Leu Arg Thr Ala Phe Ala Arg Arg Leu Glu Gly 10 aaa gtt gcc ctt ata aca gga gga gcc agt gga gtt gga gaa gtc aca Lys Val Ala Leu Ile Thr Gly Gly Ala Ser Gly Val Gly Glu Val Thr 20 gca aaa ctc ttc tcc caa cat gga gcc aaa gtt gcc att gct gat gtc 144 Ala Lys Leu Phe Ser Gln His Gly Ala Lys Val Ala Ile Ala Asp Val 35 caa gat gaa tta ggt cac tca gtt gtc gag gcc att ggc ctt tcc aat Gln Asp Glu Leu Gly His Ser Val Val Glu Ala Ile Gly Leu Ser Asn

	acc Thr													240
	gtg a Val								_	-		_		288
	aat Asn													336
	a aaa 1 Lys						-	_		_			_	384
	cta Leu 130													432
	tgc Cys													480
	tca Ser		_	_		_		_	_			•	act Thr	528
	aat Asn	Leu												576
	tct Ser													624
	gaa Glu 210													672
	aaa Lys			_			-	_	_		_	-		720
	ctg Leu			Glu										768
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Tyr Pro Asp Ser 275

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<211> 276

<212> PRT

<213> Forsythia x intermedia

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Lys Val Ala Leu Ile Thr Gly Gly Ala Ser Gly Val Gly Glu Val Thr
20 25 30

Ala Lys Leu Phe Ser Gln His Gly Ala Lys Val Ala Ile Ala Asp Val 35 40 45

Gln Asp Glu Leu Gly His Ser Val Val Glu Ala Ile Gly Leu Ser Asn 50 55 60

Ser Thr Tyr Ile His Cys Asp Val Thr Asn Glu Asp Gly Val Lys Asn 65 70 75 80

Ala Val Asp Asn Thr Val Ser Thr Tyr Gly Lys Leu Asp Ile Met Phe 85 90 95

Asn Asn Ala Gly Ile Ser Asp Pro Tyr Lys Pro Arg Val Ile Asp Asn 100 105 110

Glu Lys Ala Asp Phe Glu Arg Val Leu Ser Val Asn Val Thr Gly Val 115 120 125

Phe Leu Phe Met Lys His Ala Ala Arg Ile Met Val Pro Ala Arg Ser 130 135 140

Gly Cys Ile Ile Ser Thr Ala Ser Leu Ser Ser Thr Met Gly Gly Gly 145 150 155 160

Ser Ser His Ala Tyr Cys Gly Ser Lys His Ala Val Leu Gly Leu Thr 165 170 175

Arg Asn Leu Ala Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn Cys 180 185 190

Leu Ser Pro Phe Gly Leu Pro Thr Pro Leu Ala Lys Lys Phe Thr Gly
195 200 205

Ile Glu Asn Asp Glu Asp Leu Ala Asn Gly Ile Glu Arg Ala Gly Asn 210 215 220

Leu Lys Gly Thr Lys Leu Arg Ile Glu Asp Val Ala Asn Ala Ala Leu

225 230 235 240

Phe Leu Ala Ser Asp Glu Ala Gln Tyr Val Ser Gly Gln Asn Leu Phe
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260 265 270

Tyr Pro Asp Ser 275

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<220>

<221> PEPTIDE

<222> (1)..(21)

<223> N-terminal peptide of F. intermedia secoisolariciresinol protein wherein Xaa at positions 3, 12 and 20 represents an unidentified amino acid residue

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Ile Thr Gly Xaa Ala 20

<210> 12

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Lys

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										aaa Lys 75						240
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										gac Asp						336
ttt Phe	gaa Glu	cgc Arg 115	gtt Val	ctc Leu	agt Ser	gta Val	aat Asn 120	gta Val	acc Thr	gga Gly	gtt Val	ttc Phe 125	cta Leu	tgc Cys	atg Met	384
					Val		Ile			cgc Arg						432
										ggt Gly 155						480
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672

720

768

. 816

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2.7																
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Ser	Gln	His 35	Gly	Ala	Lys	Val	Ala 40	Ile	Ala	Asp	Val	Gln 45	Asp	Glu	Leu	
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His 65	Cys	Asp	Val	Thr	Asn 70	Glu	Asp	Gly	Val	Lys 75	Asn	Ala	Val	Asp	Asn 80	
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Ile	Ser	Asp	Pro 100	Asn	Arg	Pro	Arg	Ile 105	Ile	Asp	Asn	Glu	Lys 110	Ala	Asp	
Phe	Glu	Arg 115	Val	Leu	Ser	Val	Asn 120	Val	Thr	Gly	Val	Phe 125		Cys	Met	
Lys	His 130	Ala	Ala	Arg	Val	Met 135		Pro	Ala	Arg	Ser 140	Gly	Asn	Ile	Ile	
Ser 145	Thr	Ala	Ser	Leu	Ser 150	Ser	Thr	Met	Gly	Gly 155	Gly	Ser	Ser	His	Ala 160	

Tyr Cys Gly Ser Lys His Ala Val Leu Ala Leu Thr Arg Asn Leu Ala 165 Val Glu Leu Gly Gln Phe Gly Ile Arg Val Asn Cys Leu Ser Pro Phe Gly Leu Pro Thr Ala Leu Gly Lys Lys Phe Ser Gly Ile Lys Asn Glu 195 200 Glu Glu Phe Glu Asn Val Ile Asn Phe Ala Gly Asn Leu Lys Gly Pro 210 215 Lys Phe Asn Val Glu Asp Val Ala Asn Ala Leu Tyr Leu Ala Ser 230 Asp Glu Ala Lys Tyr Val Ser Gly His Asn Leu Phe Ile Asp Gly Gly 245 250 Phe Ser Val Cys Asn Ser Val Ile Lys Val Phe Gln Tyr Pro Asp Ser 265 <210> 24 <211> 33 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: oligonucleotide <220> <221> misc_feature <222> (1)..(33) <223> PCR primer <400> 24 acatatgcag cttcgaactg cattcgcaag aag 33 <210> 25 <211> '33 <212> DNA <213> Artificial Sequence

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